JULY, 1913. Botanical Series. Vol. VI, No. 2.

Memoirs of the Department of Agriculture in India

A SCLEROTIAL DISEASE OF RICE

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AGRICULTURAL RESEARCH INSTITUTE, PUSA

PUBLISHED FOR
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Recent investigations in plant pathology have shown that a not inconsiderable number of the diseases of plants are to be attributed to the ravages of sclerotial fungi. Considering the habitat of these parasites it is not surprising to find that tuberous crops (*e.g.*, potato, carrot, beetroot) seem peculiarly liable to attack, the disease in such cases being usually known as "root rot." In other cases, however, the host plant is attacked in the seedling stage, the symptoms closely simulating the "damping off" due to Pythium and its allies. Of sclerotial fungi, which cause such diseases, one of the best known is *Rhizoctonia*, to which the "root rot" of many tubers, as well as the "damping off" of certain seedlings, is to be attributed. There has, however, recently been detected in India a disease of rice, due to the attack of a sclerotial fungus, which seems to present some distinctive feature in its effects upon the host plant.

The fungus is known as *Sclerotium Oryzæ*, Catt., and was first described by Cattaneo (2), in 1879, as the cause of extensive damage to the rice crop in Novara and Lombardy. His description was restricted to the morphology of the fungus on the rice plant and the symptoms of disease in the crop ; as, however, culture work and inoculations were not attempted, adequate proof of the parasitic nature of the fungus was not obtained. Since the work of Cattaneo the fungus has also been discovered in Japan by Miyake (7), and has, within the past year, been collected in India.

Sclerotium Oryzæ is not, however, the only member of this genus which has been recorded as a disease of paddy. *Scl. glumale*, Ces., is known to attack rice in Borneo, while a new species, *Scl. irregulare*, is stated by Miyake to be the cause of some loss in Japan. Both these can easily be distinguished from *Scl. Oryzæ* by the size of the sclerotia. The former has been found on paddy at Noakhali, Eastern Bengal, but does not appear to be of any economic significance in India.

The genus *Sclerotium* was founded by Tode (11) in 1790, and, at present, includes a number of species of which the fertile stages are known in only a few cases. In 1816 Esenbeck (3) described six of the more important species with figures; he also includes *Thanatophytum Crocorum* (*Rhizoctonia Crocorum*) and *Erysibe suffulta* as near relatives of this genus. In a work published a few years later Fries (4) mentions fifty species and classifies them into four tribes, a sub-division which has persisted to the present day. It is interesting to note that he discards the name *Thanatophytum* for the more modern one of *Rhizoctonia*. In 1869 an enumeration of species peculiar to the Rhine district was given by Fuckel (5); an earlier publication by Kühn contains, however, more interesting matter. Kühn (6) gives some account of the work of Leveille and Tulasne (12), published a few years previously, by which the connection between *Sclerotium Clavus*, D. C., and *Claviceps purpurea*, Tul., was established. He also mentions that other species of the genus *Sclerotium* have their perfect stages among the *Clavariaceæ*. It is, of course, now well known that the original genus *Sclerotium* of Tode is an artificial one, the different members of which are really the sterile forms of widely separate fungi. *Agaricaceæ, Polyporaceæ. Clavariaceæ, Hypocreaceæ* and *Pezizaceæ* are the groups among which the fertile stages of different species of *Sclerotium* are to be found.

The Disease in the Field.

In India the first collection of *Scl. Oryzæ* was made at Noakhali. About a year later, in December 1912, fresh material

was obtained from localities as widely separate as Mandalay, Samalkota and Pusa; the external symptoms of disease were in all cases essentially the same. Infected plants can usually be distinguished from their healthy neighbours by the phenomenon of "tillering," that is to say, the development of fresh green shoots from adventitious buds at the base of the infected culm (Pl. I, Fig. 1). The infected culm gradually turns yellow and dies; any grain which it bears is light and poorly developed, in fact, there is usually nothing within the glumes. The most distinctive feature, indeed, the easiest means of detecting infected plants in the field, is the "tillering" from the base. In Pusa the disease does not make its appearance until the paddy crop is fairly well advanced; hence, even supposing that the fresh shoots remain free from the fungus, an unlikely event, there is no possibility of their producing any grain. It is the loss of grain which constitutes the most serious damage due to this fungus. In Burma the disease is one of the causes producing the condition known as "gwa-bo"; as, however, a number of insect diseases of paddy are included under this name, it is difficult to ascertain the precise amount of damage due to the fungus. The collective damage done by the combined insect and fungus attack is stated to run into hundreds of lakhs of rupees annually. Miyake in his description of the disease in Japan says: "Durch diese Parasitierung wird die Bildung der Reiskörner unvollständing, daher ist der Schaden sehr-gross." He does not appear to have observed the phenomenon of "tillering," a fact which Cattaneo also overlooked.

If a diseased culm is split longitudinally the basal portion is found to be infested with the fungus. The hyphæ form a dark greyish weft within the hollow stem, and small black sclerotia can be seen dotted all over the inner surface (Pl. II, Fig. 1). Sometimes the base of the culm is quite free from the fungus and the attack begins at a node some distance up the stem. A transverse section through an infected culm shows that hyphæ and sclerotia are not only present on the inner surface of the stem, but that the hyphæ penetrate the cells, and sclerotia are even

formed in the intercellular spaces between the main vascular bundles. This is clearly shown in Pl. III, Fig. 1. The section shows only the inner portion of the stem ; a young sclerotium can be seen in one of the larger air cavities and the collapsed nature of the innermost layer of cells, in the vicinity of the two larger sclerotia, is apparent. In some of the cells hyphæ are distinctly visible ; they are, however, more clearly shown in Pl. III, Fig. 2, from the same section. At first sight the sclerotia strongly resemble that of *Rhizoctonia Solani*, Kühn ; they are, however, considerably larger and have a distinctly smooth shiny surface. In section the younger sclerotia appear to consist of fairly small parenchymatous cells, the outer cells being more or less definitely arranged in concentric layers. (Pl. III, Fig. 1). In fact, at this stage, there is a distinct differentiation into cortical and medullary zones. In the more mature sclerotia the differentiation into cortex and medulla is not so apparent, while the cell walls are thicker and of a sharp black colour (Pl. III, Fig. 3).

Cultures.

From diseased plants, such as those just described, cultures were obtained on agar. It may be stated here that in all cases, whether the cultures were made from hyphæ or sclerotia, and whether the material came from Burma, Samalkota or Pusa, the same fungus was obtained in culture. Cultures were made on media of widely different composition, and, in some cases, the nature of the nutrient substratum was not without influence on the character of the fungal growth.

Growth appeared to be most vigorous on glucose agar. An infection upon this medium resulted in a copious development of white hyphæ, followed, after five or six days, by the appearance of sclerotia. The sclerotia are at first visible as minute circular spots of a greyish colour ; subsequently they become black and shiny, exactly resembling those found in the rice plant. The hyphæ are of the usual type, the cells being about 4—6μ broad and 150—350μ long, they contain numerous oil globules and

frequently branch. A transverse septum occurs at the point of origin of a branch and not some distance from it (Pl II, Fig. 11, cp. *Rhizoctonia*). The sclerotia are roughly circular and vary in diameter from 150—500μ. They arise from a plexus of interlacing hyphæ, which continue to branch and intertwine until a small spherical compact mass is formed. For a time the young sclerotium increases in size by the adhesion of fresh branches to the periphery; ultimately, the cell walls turn black and all further growth ceases. At this stage the interior of the sclerotium has a very definite parenchymatous structure, and it is almost impossible to discern that it has been formed by interweaving hyphæ; a thin layer of loosely intertwined hyaline hyphæ can, however, be seen investing the exterior. Not infrequently several sclerotia become united, forming an incrustation on the medium resembling a stroma; this was particularly common in cultures on maize meal.

In culture upon glucose agar, at an early stage, before the appearance of sclerotia, the mycelium along the edge of the agar appears black. An examination of the hyphæ at this region shows that they are more or less smoky coloured and of very irregular shape (Pl. II, Fig. 5); they frequently terminate in curious appendages. These appendages may consist of one or more cells; they are very much darker in colour than the cells of the hyphæ, but resemble them in their irregular form (Pl. II, Fig. 6). In some cases the parent hypha was attached to the centre of the appendage in such a way that the latter was borne in a peltate manner (Pl. II, Figs. 7, 8). Infection upon glucose agar from these hyphæ and appendages produced cultures of the normal type resembling the parent culture. The fact that these structures are produced along the edges of the agar, in contact with the glass, suggested that they might be appressoria. If this is the case, it is not easy to understand why their formation should be restricted to one sort of agar medium; in no case were they produced upon anything but glucose agar.

Infection upon nutrient agar containing extract of paddy grains gave rise to a rather different habit of growth. The

hyphæ at first spread over the surface of the agar, and slowly turn a brownish colour where they are in contact with the nutrient medium; the aerial hyphæ, however, remain white. Sclerotia arise first upon the surface of the agar, but, ultimately, may be found imbedded in it at various depths. At a level of about $\frac{1}{4}$ inch below the surface of the agar the hyphæ form a dense brownish black layer, which, upon examination, is seen to consist of an enormous number of chlamydospores. These chlamydospores are formed by the segmentation of the hyphæ into a number of short thick barrel-shaped cells. They possess thick black cell walls and contain food reserve in the form of oil drops (Pl. II, Fig. 9). Germination gives rise to a culture of the normal type.

Growth upon the special medium of filter papers was slow and produced nothing but hyphæ and sclerotia. The same may be said of French bean agar; in this latter case the fungus seemed to remain entirely superficial. Upon oat juice agar growth was very similar to that upon rice agar; in fact, it is difficult to distinguish between cultures of the same age upon these two media. Upon Lima bean agar the fungus gave rise to a curious red pigment. This developed about three days after infection and slowly spread down the tube following the growth of the hyphæ (Pl. I, Fig. 3). Growth upon maize meal was particularly vigorous, a dense white mycelium being formed in about 12 hours, followed by an abundant production of sclerotia. Here, again, the growth of the fungus is characterised by the production of red pigment in the meal.

The red pigment is strictly confined to the medium on which it is produced. If an infection is made from a culture upon Lima bean agar to glucose agar there is a faint reddening of the glucose agar just at the seat of infection, but this speedily dies away and does not spread down the tube. The production of the pigment is obviously the result of the changed metabolism conditioned by the alteration in the nutrient substratum.

Inoculations.

In order to grow the rice plants under sterile conditions the method used by Ward (13) in his investigations on the rust of wheat was employed. The seeds were first sterilised in 1% commercial formaline and then sown in sterile potato tubes containing Knop's solution. It was found that sterilisation was more efficient if performed under the air pump, the liquid, in this way, penetrating the space between the glumes more readily. Owing to the laboratory temperature in February being rather low for the growth of rice, the tubes were kept in an incubator at a temperature of 30° C. The incubator was left with its glass door facing a large north window, and, for several hours in the middle of the day, the tubes were removed and placed in the sun.

The young plants were infected when they were about 7—10 days old and about 3—4 inches high. The first series of infections was made with small black sclerotia from an agar culture about one month old. None of these inoculations gave any result, the sclerotia failing to germinate. Subsequent trials with sclerotia from old cultures showed that they had, not infrequently, lost the power of germination. Fresh inoculations were then made from a culture three days old, in which the hyphæ were still growing vigorously, and sclerotia were not yet formed. A small speck of agar was removed from such a culture and placed upon a rice culm, about 1 inch above the remnant of the seed ; hyphæ quickly spread from this centre over the exterior of the culm, which gradually lost its green colour and turned brown near the seat of infection. As the outer leaf sheath turns brown, the lamina attached to it also loses its green colour and wilts (Pl. I, Figs. 2, 3) ; finally, the process extends to the central leaves, and the whole plant dies. During the progress of the infection a light weft of hyphæ can be seen investing the culm ; in the later stages of the disease small, dense, white aggregations of hyphæ appear in this mycelium, and, ultimately, become hard black sclerotia of the usual type. This superficial production of sclerotia is a characteristic of the section Libera of this genus. We have, however, seen that sclerotia may arise in the more deep-seated portions

of the host (Pl. III, Fig 1), so too much stress must not be laid on this character. The first sclerotia usually occur about the top of the first leaf sheath, either on its inner or outer surface ; in the former case they appear as small dark swellings beneath the dry and withered leaf base. The time taken from the first infection until the death of the plant and the production of the sclerotia is about two weeks.

Portions of dead and dying plants, some of which had not yet produced sclerotia, were incubated in agar tubes. In all cases they gave cultures of *Sclerotium Oryzæ*, which exactly resembled those from which the inoculations had been made. Since the infections showed that the fungus was strongly parasitic, and could penetrate the uninjured external surface of the plant, it was not considered necessary to make wound inoculations. On the whole, 70—80% of the inoculations proved fatal.

Microscopic examination showed that the behaviour of the mycelium in the infected culm was not without interest. The

bulk of the hyphæ appear to run longitudinally in the large air cavities of the leaf and in the cells bordering on them (Pl. II, Fig. 11), while a certain number grow outside the leaves in the spaces between the folds of the lamina ; these latter are particularly obvious investing the delicate edges of the inner leaf (see text figure). It is at spots such as this that the rice plant is

peculiarly liable to infection by the fungus. On the outer, dorsal, surface of the leaf sheath the epidermal cell walls are much thinner in the areas between the vascular bundles than they are immediately behind them, and, at the edges of the leaf, they are extremely delicate. Moreover, immediately behind the vascular bundles, not only the epidermal, but two or more hypodermal layers have thickened walls, which give a cellulose reaction with chlorzinc iodide (see text figure). On the ventral surface of the leaf sheath the epidermis is uniformly thin, and does not show any such differentiation. Thus, on the outer surface of the leaf sheath, there are areas which offer a poor resistance to any parasitic attack. Once the fungus has gained an entry at one of these spots, progress to the more delicate and deeper seated tissues is easy.

The hyphæ are both inter-and intra-cellular. It is by no means unusual to find that a hypha which has penetrated a cell wall possesses a pronounced thickening in the portion on that side of the cell wall from which penetration has taken place (Pl. II, Fig. 11). This swelling may be taken as evidence of the increase in chemical activity, probably in the direction of the secretion of enzymes, which precedes the solution and penetration of a cell wall.

A distinction must be noted here, between the behaviour of the infected plants used in the inoculation experiments, and the course of the disease in the field. A successful inoculation killed the infected plant completely (see Pl. I, Fig. 3); but, in the field, the result of an attack seemed rather to be a gradual weakening of the host, culminating in the failure to produce good seed. Further experiments on a field scale are necessary to elucidate this point. It may perhaps be the case that rice plants grown under the sterile conditions of our experiments were less capable of resistance to the disease than under normal circumstances

Conclusion.

The above account differs in several important respects from that of Cattaneo. In particular the Italian author describes

some unusual structures in the sclerotium. He states, that when the sclerotia are about a fortnight old several vacuoles make their appearance in the interior, and, ultimately, coalesce to form one large central space; the sclerotium thus becomes hollow. From the solid exterior hyphæ now grow into the central vacuole and bear spores. The spores are circular structures, about 12μ in diameter, and are either borne terminally, or else laterally, on the walls of the hyphæ. Such a method of spore formation was never observed in our cultures, nor did a series of microtomed sections reveal a sclerotium as anything but a solid pseudoparenchymatous structure. The only bodies bearing any resemblance to the spores of Cattaneo were the oil drops in the hyphæ. By crushing a sclerotium the hyphæ become torn and the oil drops set free. Under these circumstances the oil drops may adhere to the sides and ends of hyphæ, in positions not unlike those which Cattaneo figures for the spores.

No trace of a perfect stage was ever observed. Brefeld (1) has pointed out that the sclerotia of certain *Basidiomycetes* (*Agaricus, Coprinus, Typhula*), and of *Penicillium* and *Erysiphe*, arise by the interlacing of branches of a single hypha, while those of the genus *Peziza* are formed rather from a plexus of interwoven filaments. This comparison has been somewhat erroneously generalised by some writers (Stout 10) into the statement that the sclerotia of *Basidiomycetes* arise from a single hypha and those of *Ascomycetes* from a plexus of hyphæ. In the genus *Rhizoctonia* it has been found (Shaw 8) that *Rhizoctonia Solani*, Kühn, forms its sclerotia from a single hypha, whereas, in the macro-sclerotial species, of which *Corticium vagum* is the perfect stage, the sclerotia arise from a mycelial plexus. It is evident, therefore, that this character does not afford any basis for taxonomic consideration, since both methods of sclerotial formation occur in both *Basidiomycetes* and *Ascomycetes*.

It is interesting to note the changes in the appearance of the fungus, according to the nature of the nutrient substratum. These changes are most marked in the colour and form of the

hyphæ, and, as Stevens and Hall (9) have already pointed out, afford ground for reflection when we consider the characteristics on which some groups of the *Fungi imperfecti* are classified.

Against a parasite such as *Scl. Oryzæ* it is difficult to see what remedial measures can be employed with success. The sclerotia of the fungus undoubtedly perennate in the soil, where, under favourable conditions, they germinate and produce a mycelium which attacks the paddy crop. Cattaneo suggests the application of salammoniac, with a view to killing the sclerotia in the soil. Even if this is successful on a small scale, it is manifestly impossible to the extent which would be necessary in India. Probably the breeding of resistant varieties is the only method by which any permanent resistance could be made in the case of a field crop such as rice. Fortunately the damage done in India at present does not appear to be sufficient to bring this question within practical consideration.

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APPENDIX.

GLUCOSE AGAR.—

Extract of Lemco	4 grms.
Sodium chloride	5 ,,
Peptone	10 ,,
Glucose	20 ,,
Agar	15 ,,
Water	1000 c.c.

RICE AGAR.—

Take 50 grms. of crushed rice seed and boil with 300 c.c. of water for 1 hour, strain through a wire gauze. Dissolve 10 grms. agar in 200 c.c. water, add the decoction and heat to mix thoroughly.

Oat Agar, Lima bean agar and French bean agar are prepared in the same way with the substitution of oats, Lima bean, or French bean for the rice grains.

FILTER PAPER.—

Ammonium nitrate	10 grms
Magnesium sulphate	1 ,,
Potassium phosphate	5 ,,
Lactic acid	2 ,,
Water	1000 c.c.

Take 50 c.c. of above solution, add 10 grms. filter paper and sterilise.

DESCRIPTION OF PLATES.

PLATE I.

FIG. 1.—Rice plant infected with *Sclerotium Oryzæ*—note the young shoots growing out from the base of the infected culm and the outer sheathing leaf covered with sclerotia. × 1.

FIGS. 2, 3.—Young plants inoculated with *Sclerotium Oryzæ.* In 3 sclerotia are forming. × $\frac{2}{3}$.

FIG. 4.—Culture on Lima bean agar. × 1.

PLATE II.

FIG. 1.—Rice plant with *Sclerotium Oryzæ*—note sclerotia formed within hollow stem. × $\frac{4}{3}$.

FIGS. 2, 3, 4.—Young and old sclerotia from glucose agar culture. × 50.

FIG. 5.—Hypha from the edge of glucose agar culture. × 700.

FIG. 6.—Appendage on hypha. × 700

FIGS. 7, 8.—Appendages borne in peltate fashion. × 700.

FIG. 9.—Chlamydospores from rice agar culture. × 700.

FIG. 10.—Hypha from rice agar culture breaking up into chlamydospores and single cells. × 700.

FIG. 11.—Longitudinal section of leaf showing hyphæ in intercellular space and cells bordering on it. × 700.

PLATE III.

FIG. 1.—Microphotograph. Transverse section of stem of diseased rice plant from Pusa crop. Note two sclerotia present on the inner surface of stem and one small sclerotia in large air cavity. × 50.

FIG. 2.—Microphotograph. The same showing hyphæ in cells. The rounded bodies with dark centres are starch grains. × 170.

FIG. 3.—Microphotograph. Section through mature sclerotium. × 130.

PLATE I.

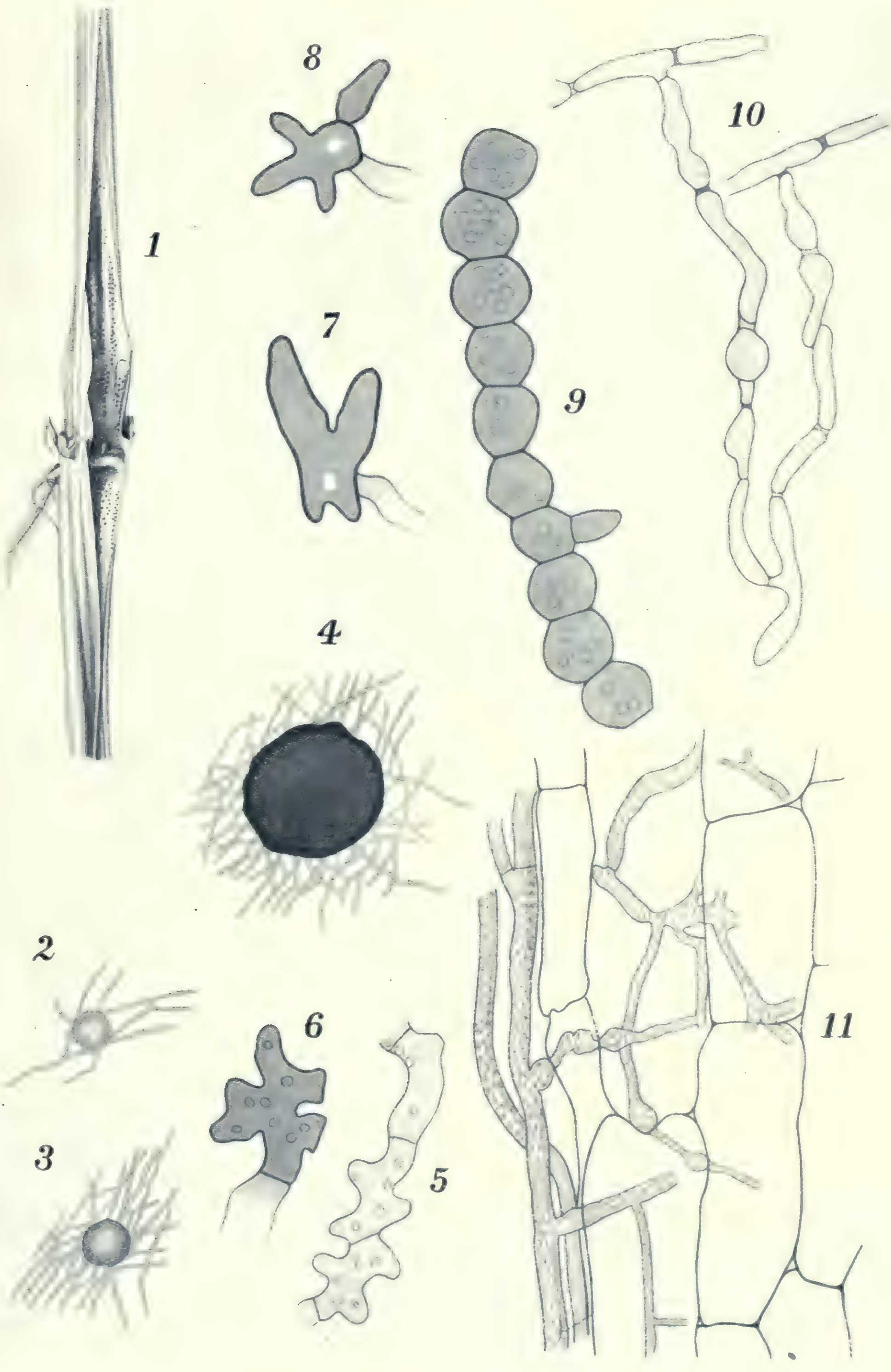
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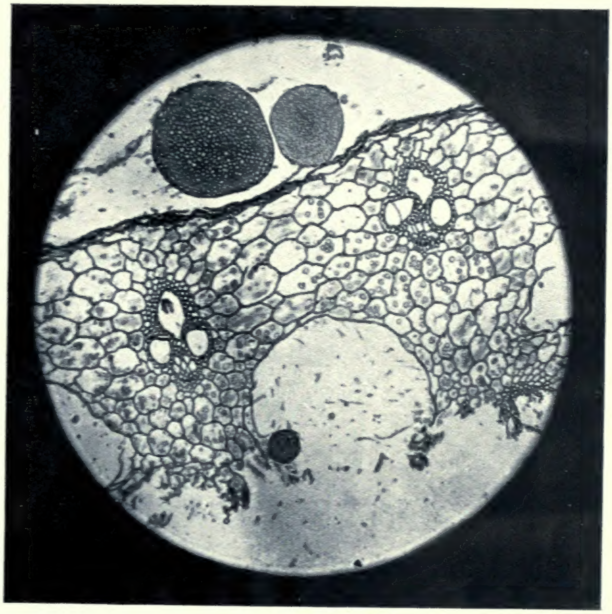
PLATE III.



Fig, 1.

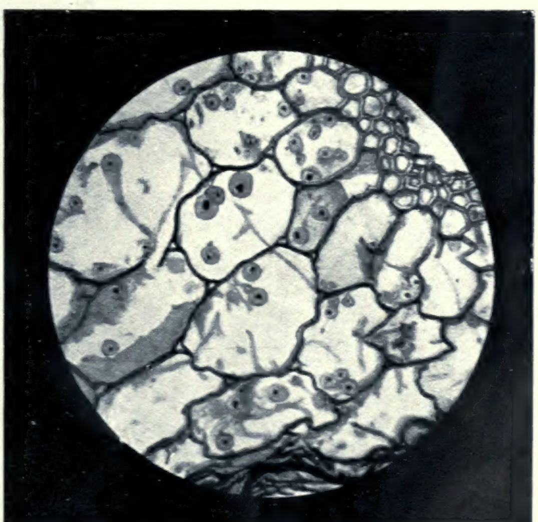


Fig. 2.

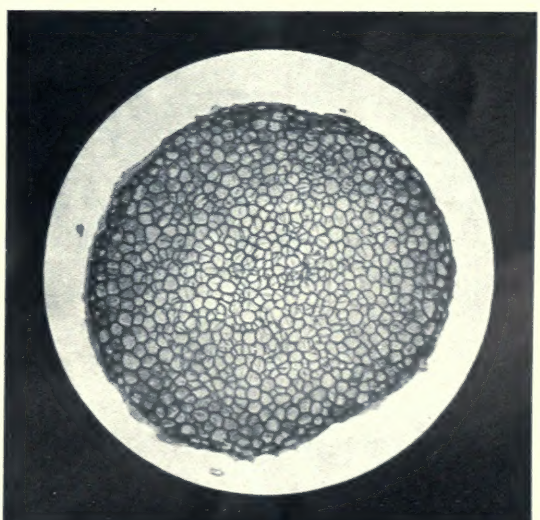


Fig. 3.

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